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A Comparison of Extraction Methods for Terrestrial Arthropods

C.A. EDWARDS & K.E. FLETCHER[1]

I

Introduction

Studies of the productivity of terrestrial communities made as part of the International Biological Programme, will deal with energy flow, nutrient cycling, trophic structure, spatial patterns, relations between species and the diversity of species of plants and animals in chosen ecosystems. For many of these studies the numbers of plants and animals in different communities must be accurately assessed. Plants, the primary producers, can often be counted simply, and although populations of larger animals, the principal secondary producers, are more difficult to estimate, many do not present insuperable difficulties. Populations of small invertebrate animals may contribute a substantial part to the total biomass of the soil ecosystem and to count them is often very difficult, for they are very numerous and their numbers must be estimated by sampling. The number and size of the samples needed varies and depends on the size, activity and distribution of each species. Sampling areas of soil to estimate the numbers of invertebrate animals has been adequately discussed by Hartenstein (1961), Healy (1962), Debauche (1962), Skellam (1962), Hughes (1962) and Macfadyen (1962a). Sampling for particular groups will be dealt with elsewhere in this handbook, as, for example, the detailed treatment of Acari in the chapter by Berthet.

No method yet used, successfully extracts all the invertebrate animals from a soil sample. The immediate aim is to extract as many as possible, and to standardize methods for comparative studies.

This paper describes how the efficiency of all the more common methods of extracting invertebrate animals from soil, in different habitats and soil types, were compared on a scale large enough to assess the influence of the many factors that cause methods to differ in efficiency. From the results the most

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suitable methods may be recommended. Methods range from simple sieving or hand-sorting to use of complex pieces of apparatus. The principal methods are of two kinds (Murphy, 1962): those by which the animals are driven from soil, are called dynamic methods; and those by which the animals are physically separated from the soil, i.e. the mechanical methods.

Different stimuli used to encourage the animals to escape from the soil include chemical attractants and repellants, electrical stimuli, vibration and heat, but all the common methods repel the animals from soil samples by changes in moisture content or temperature. The other methods are too specific and too inefficient to merit fuller consideration here.

The earliest funnel method developed by Berlese (1905) consisted of a double-walled funnel containing hot water. The soil or litter rested on a wire mesh and the emerging animals fell into a tube beneath. This funnel was much less efficient than that described by Tullgren (1918) which used heat from a light bulb suspended over the funnel. Most funnels now use Tullgren's principle although with many modifications. For instance, Ford (1937) used a coiled hot wire for the heat source, and Haarlov (1947) showed that a gap between the edge of the sieve and the wall of the funnel prevented water condensing on the walls and trapping the animals after they emerged from the soil. Some of the early Tullgren extractor funnels used ordinary glass laboratory funnels, but Murphy (1950) and others showed that steep-sided funnels were better and Macfadyen (1962) even abandoned the funnel and collected the animals directly into a tube of the same diameter as the sieve that supported the soil. The earlier funnels were large, but there is now a tendency to use many small funnels to allow adequate replication of samples.

There have been many attempts to minimize the amount of soil and debris that falls into the collecting tube (Ulrich, 1933; Valle, 1951; Newell, 1955; Murphy, 1958) but few succeeded enough to be adopted generally, because they also usually lessened the efficiency of extraction. Macfadyen (1953) and Kempson *et al.* (1963) constructed very sophisticated apparatus which completely isolated the upper and lower surfaces of the soil sample and, by cooling and humidifying the lower surface, maintained steep gradients of humidity and temperature in the sample.

The simplest mechanical method of separating small animals from soil is by hand-sorting. This is tedious and inefficient, even when augmented by sieves. Most mechanical methods now rely on differences in density between the animals and the soil, or on the lipophilic properties of cuticles. Impregnated soil samples have also been sectioned and animals counted at the surface, but this method is laborious and unlikely to provide enough data for adequate statistical analysis.

The specific gravity of most small invertebrates lies between 1·0 and 1·1 (Edwards, 1967b) and, as the cuticle is hydrophobic, many animals float from soil stirred in a container of water. When solutions such as brine, sugar or magnesium sulphate, with a specific gravity of about 1·2, are used instead of water, all the animals that are not trapped in the soil float to the surface and can be decanted. Most of the flotation methods in common use are based on this principle. The percentage of animals recovered can be considerably increased by washing the soil through a series of sieves of decreasing mesh size to separate out stones, organic matter and plant material, and break down the soil particles (Morris, 1922). Ladell (1936) further refined the method by agitating the contents of the final container (commonly called a 'Ladell' can) and by bubbling compressed air through the particulated sample immersed in magnesium sulphate solution. This freed the animals from soil and considerably increased the efficiency of the method. Salt and Hollick (1944) described a method and apparatus using these principles that has been widely used with only slight modifications. They added a final stage designed to separate the animals from the organic debris that also floats to the surface of the dense solution, namely shaking the debris and animals with a mixture of xylene or benzene and water. The animals were coated with the organic solvent and, when this rose to the surface, they lay within it just above the water and could easily be picked off from the plant debris, which lay just below the interface in the aqueous phase.

This method is extremely laborious and the efficiency of operators differs. To speed the process, Edwards and Heath (1962) mechanized the washing stage by rotating the Ladell can and sample in a large mesh sieve under a vertical flat fan jet of water. Animals were extracted from the washed soil by shaking soil, debris and animals with a 50/50 mixture of zinc sulphate solution (specific gravity, 1·4) and a mixture of xylene and carbon tetrachloride (specific gravity 1·2), in a specially constructed polythene separating funnel. The animals floated to the surface of the organic solvent and the plant debris to the interface between the zinc sulphate solution and organic mixture; the soil sank to the bottom. The soil and plant debris were then run off leaving the animals in the funnel. Alternatively a siphon system was used to raise the level of the organic mixture until the animals overflowed through a side arm.

Aucamp and Ryke (1964) also used the lipophilic properties of the arthropod cuticle. Small samples of soil were placed in water in perspex containers with sliding plates coated with lanolin, silicone grease or petroleum jelly. After rotating the containers for fifteen minutes the animals stuck to the grease could be examined and counted under a binocular microscope.

Some workers separate invertebrate animals from the soil and debris by centrifuging. Usually air is boiled from the sample which is then centrifuged in a dense solution that supports the animals but not the soil and debris. These methods are suitable only for small soil samples.

Flotation methods are believed to extract a greater proportion of invertebrate animals than dynamic methods, but specimens are often badly damaged and unidentifiable; flotation also collects both dead and live animals and cannot be used for soils with much organic matter. However, flotation methods are much better than dynamic methods for clay soils and allow samples to be stored cold until there is time to deal with them.

Dynamic and flotation methods were compared by Evans (1951), Macfadyen (1953), Edwards (1955), Satchell and Nelson (1962), Wood (1965) and Block (1967), who all compared two methods on a single site or soil type and often had experience of only one of the methods. Such comparisons may be misleading because a method suitable for one type of soil may be unsuitable for another. Flotation methods are unsuitable for enchytraeid worms and nematodes, animals that are only poorly wetted in the oil/water stage.

The efficiency of different extraction methods range from near zero to close to 100% for any particular group of animals, and none is completely successful with all soils and animals. The absolute number of animals in a soil sample is not known, and there is no known way of extracting them all; therefore the efficiency of extraction is difficult to assess. Some workers have put known numbers of animals into soil and then extracted them by different methods, but the animals do not penetrate the soil completely and they are extracted more easily than from soil they were inhabiting naturally.

To compare all the common methods of extraction for different habitats and soil types was beyond our resources and the following studies were made as a compromise. Many soil ecologists were asked for details of their methods and on the basis of their replies, eleven commonly used extraction methods were tested with soil from woodland, pasture and arable land all on the same soil type. The efficiency of extraction of the commonest dynamic and flotation methods was compared using five different soil types ranging from sands and peats to clays. Factors that influenced the efficiency of a standardized form of the Tullgren apparatus were also studied.

The information which showed the best methods for each group of animals in particular habitats or soil types is given in the last section of this paper. The complexity of the different methods, their availability and practicability are also discussed. Definite advice is given about methods for use in the PT section of the International Biological Programme.

II

Analysis of questionnaires

Of 150 questionnaires sent out to soil zoologists and ecologists, 82 were completed and returned. Questions were asked about general methods of extraction, about those used to study particular groups and about details of apparatus and methods and any major modifications. For example, questions were asked about the size and shape of Tullgren funnels, the power and distance from the sieve of the source of heat, whether the funnels were cooled, the time taken for complete extraction, and the preservative liquid in the collection vials. Details of flotation methods were asked for, such as the nature and specific gravity of the dense solution and whether an oil/water stage was used.

Replies were sometimes incomplete, sometimes more information was given than was requested, but we thank all who answered. The frequency with which different methods are used is summarized in Fig. 1. Most workers used a single method; 74% used some form of Tullgren funnel and 61% of these used a relatively standard kind, usually with electric light as a heat source. The few that used neither light nor heat were mostly concerned with taxonomy. Seven workers used modified Macfadyen funnels with steep gradients of heat and humidity produced by cooling and humidifying the completely separate lower part of the sample.

Four workers used simple flotation with dense solutions for general studies, but several others used flotation with sugar solution for Collembola and other fragile animals which were recovered in good condition for taxonomic studies. Except for dynamic methods Salt and Hollick flotation was by far the most popular method (12%). Several workers hand-sorted the soil for the larger animals, either in the field or in the laboratory. Most of the other methods were used only by those workers who devised them; for example, the Muller and Naglitsch centrifugal flotation method, d'Aguilar flotation, and grease film extraction.

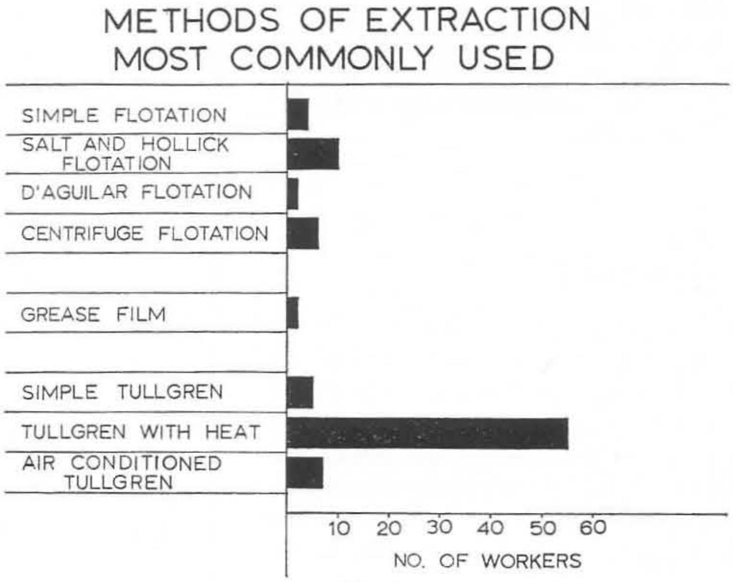
METHODS OF EXTRACTION
MOST COMMONLY USED
SIMPLE FLOTATION
SALT AND HOLLICK FLOTATION
D'AGUILAR FLOTATION
CENTRIFUGE FLOTATION
GREASE FILM
SIMPLE TULLGREN
TULLGREN WITH HEAT
AIR CONDITIONED TULLGREN
10
20
30
40
50
60
NO. OF WORKERS

FIG. 1

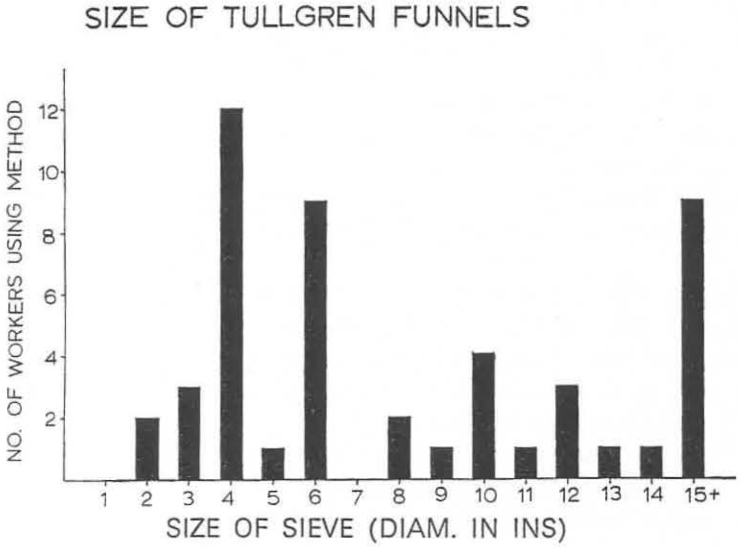
SIZE OF TULLGREN FUNNELS
NO. OF WORKERS USING METHOD
12
10
8
6
4
2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15+
SIZE OF SIEVE (DIAM. IN INS)

FIG. 2

The Tullgren apparatus and the method of using it varied greatly (Fig. 2). Funnels ranged from 5 cm to more than 37·5 cm in diameter, but 10 cm was the most common diameter and 15 cm diameter funnels were often used. Small funnels and more samples are becoming more popular.

Workers were asked whether soil samples were placed in the funnels with as little disturbance as possible or were broken up, and of seventeen who answered, nine did not disturb the samples (Fig. 3). The power of heat source varied (Fig. 4); most workers used electric light bulbs of less than 35 watts, but some used 100 watts and one 200 watts, so the time taken to complete the extraction (Fig. 5) consequently ranged from 1 day to more than 12 days.

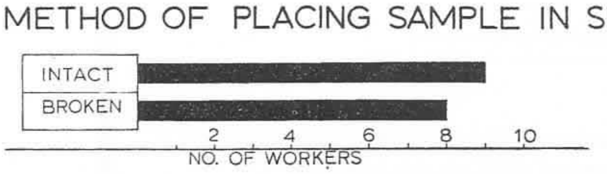

FIG. 3

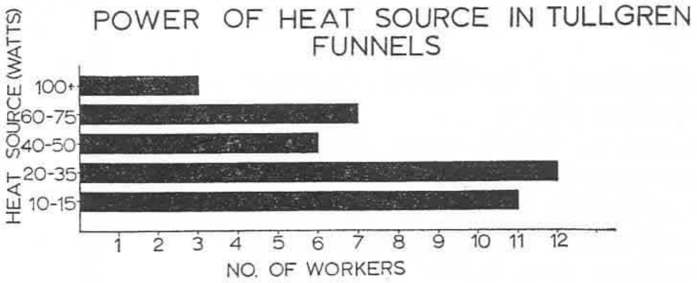

FIG. 4

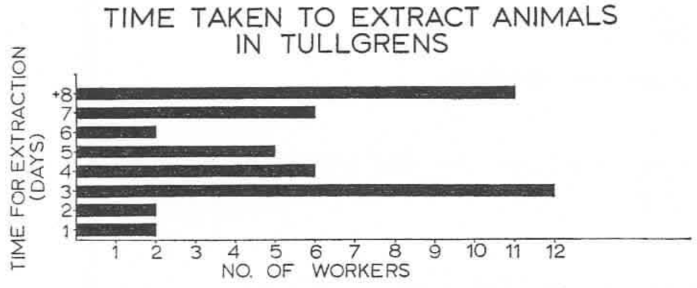

FIG. 5

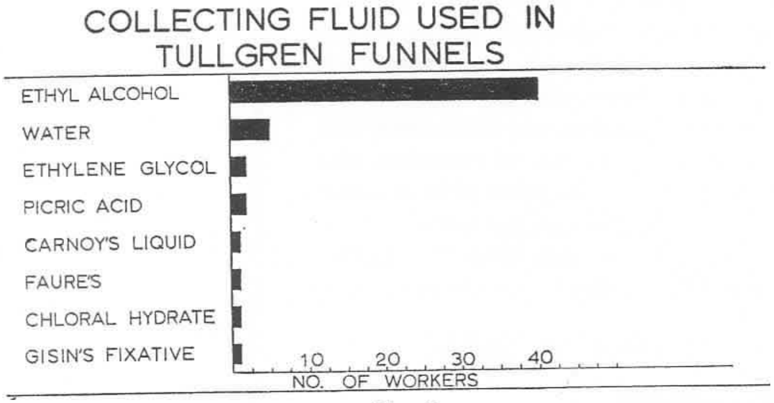

FIG. 6

Most Tullgren funnels took either 3–4 days to extract all the animals or more than seven. Many workers taking longer to extract their samples claimed a more efficient extraction. The replies suggest that many workers extracted for an arbitrary time without fully testing whether it was adequate for all soils they investigated.

Most workers used ethyl alcohol in the collecting vials under the funnels, a few used water and a minority, other fixatives (Fig. 6).

The questionnaires enabled us to choose the methods to test. In general, dynamic methods were preferred, probably partly because they were easy to operate and because specimens were undamaged. Our studies showed that funnels are comparatively efficient, and because most participants in the International Biological Programme will use them, factors affecting their efficiency were studied.

III

Description of extraction methods tested

(1) Dynamic methods

These are all based on the original Tullgren principle but differ enough to justify testing five versions in several ways.

(A) *The simple plastic funnel extractor*

Sixteen domestic plastic funnels are mounted upon two plywood boards set one above the other with holes to support each funnel. The funnels, 16·5 × 19·8 cm deep, have an included angle of 60°, and narrow to an opening 1·2 cm diam., where the collecting vessel, 2·5 cm diam. × 5 cm deep is held in position by a rubber bung. The soil samples are contained in anodized aluminium sample containers, 12·8 cm diam. × 7·5 cm deep, and rest upon a 10 mesh, 23 S.W.G. stainless steel sieve. The sieves are held in position by wire supports from three outwardly directed lugs 1·2 cm long, at the base of the sample container. The samples were extracted for nine days, at a room temperature thermostatically maintained at about 20°C and the animals were collected into vials containing 70% v. aqueous ethyl alcohol and glycerol (in the ratio 20: 1).

(B) *The Rothamsted funnel extractor without light or heat source*

This battery of sixteen anodized aluminium funnels is mounted upon two plywood boards, set in an angle-iron framework, one above the other, with holes 12·8 cm diam, and 4·2 cm diam., respectively, to support each funnel. The funnels 15 cm diam. × 25 cm deep, with an included angle of 32°, narrow to an opening 1·2 cm diam., where a collecting vial, 2·5 cm diam. × 5 cm deep, is held in position by a rubber bung. The soil samples rest on a 10 mesh S.W.G. stainless steel sieve in anodized aluminium sample containers, 12·8 cm diam. × 7·5 cm deep. The sieves are held in position by wire supports from four outwardly directed lugs, 2·5 cm long, at the base of the sample container. The sample container is supported in the top of the funnel by the lugs, leaving a gap between the sieve and funnel that minimizes condensation. The samples were extracted for nine days at a room temperature thermostatically maintained at 20°±1°C. The animals were collected into vials containing 70% v. aqueous ethyl alcohol and glycerol (in the ratio 20: 1).

(C) *The Rothamsted funnel extractor with light and heat source*

The apparatus is similar to that in B above but over each sample a tinned cylinder 26·6 cm high × 12·8 cm diam. is suspended, containing a 25 w. pearl electric light bulb 25 cm above the surface of the soil. The light bulbs, wired in parallel from a 240 v. mains were controlled by a rheostat. The extraction time was 5 days. The temperature of the soil nearest the bulb increased from about 35°C after the first 12 hours to about 39°C after 5 days. The room temperature was controlled at 20°±1°C during the whole of the extraction period.

(D) *The Murphy split funnel extractor* (Murphy, 1958)

This apparatus has 18 small funnels mounted on a wood and metal framework, and a similar battery of 18 light cylinders suspended above. The cylinder unit is counterpoised and can be raised for access to the funnels and lowered to cover the sample containers. The funnels, 8 cm diam. × 24·2 cm, narrow to an opening 1·6 cm diam., where a collecting tube, 2·5 cm diam. × 5 cm deep, is held in position by a rubber bung. The sample container, 5 cm diam. × 5 cm deep, has three brackets at its base supporting a sieve plate, 14 mesh, 26 S.W.G., held 0·5 cm below the lower rim of the sample container leaving a gap at the sides. This unit is supported on a large sieve plate, 4 mesh, 20 S.W.G., which rests at the base of the upper portion of the funnel. The cylinders 7·5 cm diam. × 30 cm deep each contain a 15 w. electric light bulb. The soil samples, 5 cm diam. × 5 cm deep, were extracted intact and inverted with vials containing 70% v. ethyl alcohol and glycerol (in the ratio 20: 1) below the funnels. The extraction took six days in a room at a temperature of 16–20°C with the air temperature at the top of the sample about 30°–35°C.

(E) *The Kempson infra-red extractor* (Kempson *et al.*, 1963)

The apparatus uses a similar principle to the Macfadyen canister but is much larger. It consists of a rectangular cabinet of fibre board open at the top, supported by a framework of slotted angle. A fibre baffle board separates the cabinet into two compartments. In the lower compartment a fibreglass tray, 90 cm × 54 cm, is used as a continuous-flow cold water bath. In the upper compartment two 250 w. infra-red lamps, are suspended 51·5 cm above the baffle board and these are wired to a 'Sunvic Simmerstat' time switch (type TXY–B) and to two 100 w. light bulbs, so that when the infra-red lamps are off, the 100 w. bulbs are on. The sample holders are round plastic dishes, 12·5 cm diam. × 5 cm deep, with tapered sides. The bottom of each dish, cut away and replaced by a grid of stiff plastic with approximately 1 cm mesh, fits into holes carefully cut in the fibre baffle board. Below each sample container there is another plastic bowl joined to it by a tightly fitting rubber ring and containing saturated picric acid solution. Access to the sample holders is through a vertically sliding door in the front of the cabinet. The bowls containing the collecting fluid are partly immersed in the cold water bath and the soil samples are placed upon a circle of cotton fillet net (11 threads per 2·5 cm each warp and weft) supported by the plastic grid. The gap between the sides of each soil sample and the sides of the container is filled with expanded polystyrene with holes cut to fit the sample to reduce heat transfer between the upper and lower compartments. On the 3rd day of extraction the simmerstat was switched on at a low setting. During the fourth and fifth days the heating was increased and by the end of the sixth day the lamps were left on all the time until the end of the extraction on the eighth day. Temperatures at the soil surface were about 70°C at the end of the extraction period.

(F) *The Macfadyen high gradient canister extractor*

The apparatus, based on Macfadyen's (1962b) high gradient canister extractor as described by Block (1966), does not use funnels. Thirty-two extractor units arranged in four rows of eight, each contain a cylindrical soil core 3·8 cm diam. × 3·0 cm deep. The units are held in two trays which form the water baths for cooling the collecting canisters. A horizontal asbestos baffle separates the heating from the cooling compartment. The intact soil cores are inverted over a metal gauze (25 mesh per cm^2) on the top of an aluminium canister 4·0 cm diam. × 8 cm deep containing the collecting fluid. The sample holder of heat-resistant laminated phenolic plastic 'Tufnol', is fixed to the top of the canister by a watertight rubber sleeve. The units are held in position by holes in the asbestos baffle. A steep temperature gradient is maintained throughout the soil sample by heating from above by a 60 w. pearl electric light bulb, and cooling the collecting canisters below by circulating cold-water through the bath. A 'Zenith' variac transformer (type 100M) controls the voltage passing through the electric light bulb above each unit. The animals drop into distilled water. Samples were extracted for three days, with heating controlled at the following voltages: 0–24 hours at 60 volts, 24–48 hours at 100 volts and 48–72 hours at 140 volts. The temperature at the sample surface was approximately 120°C at the end of the extraction period.

Temperature changes

	Sample		
Time	Top	Bottom	Gradient
24 hrs	32°C	20°C	12°C
48 hrs	70°C	30°C	40°C
72 hrs	120°C	50°C	70°C

(G) *The Macfadyen air-conditioned funnel extractor* (Macfadyen, 1962b)

The apparatus is completely enclosed in a rectangular cabinet, made of chipboard, supported on an angle-iron framework, and lined with expanded polystyrene. The funnels arranged in rows of three, fit tightly into holes in removable chip-board trays and are 25 cm high × 15 cm diam. with an included angle of 32° narrowing to an opening 1·2 cm diam. Collecting tubes 2·5 cm diam. × 5 cm deep are held in position at the bottom of the funnels by rubber sleeves. A door in the front gives access to the funnels in the front and the sample containers, 10·5 cm diam. × 5 cm deep, are suspended above funnels, and mounted in a baffle board covered with aluminium foil to minimize heat transfer. The sample containers are supported by a metal rod across the top of each funnel and held firmly in position by the holes in the baffle. The upper part of the cabinet containing the heater assembly is supported on pulleys and can be raised to insert samples and lowered onto the sample containers, which fit flush to the surface of the baffle board. The heaters are lengths of 20 gauge nichrome wires 7·5 ohm stretched between ceramic insulators. Each heater serves three funnels and are arranged in pairs each side of vertical ducts. A fan circulates moist air in the lower compartment with ducts arranged so the air currents pass over a cold water bath, through the coils of an evaporator from a refrigerator, to the lower surface of the soil sample. The air then passes through holes in a false back plate and back to the fan. The air passages in the false back are of equal length, to ensure an even air flow to all funnels. The evaporator coils lead to long lengths of flexible copper pipe to complete the circuit between the compressor and condenser. The compressor is driven by a ¼ h.p. capacitor start induction motor (230 v. 50 cycles single phase) and a thermostat controls the air temperature in the cabinet.

The soil samples were taken in the field in the sample containers, which had a sieve of 10 mesh, 23 S.W.G., held in position by cross-wire supports from the sides. Intact samples were inverted and left in the apparatus for nine days and animals were collected in 70% v. aqueous ethyl alcohol and glycerol (ratio 20: 1). For the first seven days the air in the lower cabinet was regularly moistened, using an atomizer driven by a compressor to maintain a relative humidity greater than 85% and cooled (8–10°C) while the temperature in the heating compartment increased to 45°C. The refrigerator unit was switched off on the seventh day and the relative humidity allowed to fall.

(2) Mechanical methods

(H) *Simple brine method of flotation*

The soil sample, immersed in a saturated sodium chloride solution (S.G. 1·2) in a galvanized can is stirred until most of the soil is in suspension in the solution; it is then allowed to settle, and the floating material decanted into a 150 mesh sieve container. The process is repeated to ensure all floating material is collected. The collected material is washed free from brine solution and carefully transferred, together with some water, to a 500 ml conical flask. Xylene is added and the mixture shaken. The contents of the flask are poured into a 1 litre beaker and the xylene-water interface brought to the top of the beaker by adding more water. The interface is examined under a binocular microscope and the animals picked out with a small wire spoon.

(I) *The Salt and Hollick method of flotation*

The apparatus is modified slightly from that described by Salt and Hollick (1944). The soil samples are put in cans and water added. They are then frozen at −10°C to break up the soil particles and kept at this temperature. Before flotation each soil sample is thawed and the soil washed under high-pressure jets of water through two sieves with successively smaller mesh into a reservoir below. The meshes of the two sieves are 7 mm and 2·5 mm respectively. Material retained by the 7 mm mesh is washed, teased apart if necessary and discarded and fragments retained by the 2·5 mm mesh are washed into the reservoir. The excess water and suspended material overflow into another deep container with an overflow lip and a 120 mesh sieve in the bottom (43 S.W.G.) ('Ladell' container). The water and fine silt particles pass through the 120 mesh sieve and run out to a drain. After all material is washed from the sieves the first container is tilted and the contents washed into the 'Ladell' container, which is then drained and placed on a pivoted stand. A saturated solution of magnesium sulphate (S.G. 1·2) is used to float the organic material to the surface. Compressed air, passed through the base of the 'Ladell' container, agitates the soil and free organic particles.

The material floating to the surface is decanted into a cylindrical glass tube, diam. 5 cm × height 17·5 cm, covered at the base with a piece of 180 mesh bolting silk. The organic matter collected is washed and shaken in a flask with water and xylene. The animals are picked off the xylene-water interface under a binocular with a small wire spoon.

(J) *The Edwards and Heath method of flotation*

This apparatus (Edwards and Heath, 1962) is a mechanized Salt and Hollick apparatus. The 'Ladell' container is of black polythene with a 180 mesh sieve at the bottom. Four of these modified 'Ladell' containers rest in rotating funnels driven by a common shaft geared to a ¼ h.p. electric motor so they rotate on the vertical axis at 20 r.p.m. The soil samples, stored at - 10°C, are thawed and placed in wire mesh (4 mesh, 23 S.W.G.) baskets standing on the 'Ladell' sieve. Each soil sample is sprayed with water from two fixed flat fish-tail jets. One plays across the base sieve to prevent it blocking with silt and the other is directed vertically downwards to break up the soil as it rotates. The water and fine silt particles pass through the sieve, along channelways to a settling tank, and then to the drain. The water jets are fixed to a counterpoised bar, which can be raised to allow the 'Ladell' containers to be removed and samples replaced. Each soil sample was washed for 10–30 min. according to soil type, and any material retained in the wire basket was washed above the 'Ladell' container, teased apart, if necessary, and discarded. The subsequent procedure was similar to the Salt and Hollick method, with a flotation, and oil-separation stage.

(K) *The grease film method of extraction* (Aucamp and Ryke, 1964)

This apparatus consists of two rectangular plastic tanks (7·5 cm × 10 cm × 20 cm) each containing two removable plastic plates 20 cm × 10 cm which slide into tracks in the tank so they are held against the inner surfaces. These plates are coated on the inner surface with a thin layer of silicone or other grease such as Shell white petroleum jelly. The soil samples (5 cm × 5 cm), stored at - 10°C, are thawed, and allowed to stand in beakers of water, with occasional stirring, for 1 hour. This breaks up the soil particles and separates any tangled root present. The water-soil mixtures are poured into the two plastic tanks and water added to make them of equal weight. A lid, fitted onto each tank, was kept watertight by a rubber seal and clamp. The tanks are then fitted into a frame rotating about its horizontal axis and which can be tilted to a vertical position to facilitate loading. The frame rotates on a central axis, driven by an electric motor at 16 revolutions per minute and samples are rotated for 15 minutes. The grease plates are removed and examined under a binocular microscope and most animals can be identified while still embedded in the grease.

IV

A comparison of all methods on a single soil type

The methods described in Section III were compared in woodland, pasture and arable habitats all on a silt-clay loam soil. From each site, samples were taken with the appropriate coring tools as close to one another as possible, from a small uniform area, and were immediately placed in aluminium tins and taken to the laboratory. Randomly selected batches of sixteen were placed in each of the sets of funnels; the remaining samples, for extraction by flotation and grease-film, were sorted into similar batches and stored at −10°C. Sixteen samples are the minimum number required to show a difference in efficiency of 20% to be significant between methods for most species of the micro- and meso-fauna: more are needed for sparse or aggregated animals. Samples were taken only to a depth of two inches because more than 80% of the arthropods were in this layer.

KEY TO FIGURES 7-16

DYNAMIC: A. Plastic Funnels B. Tullgrens (no heat source) C. Rothamsted Tullgrens D. Murphy Split Funnels E. Kempson Infra-red F. Macfadyen Canisters G. Macfadyen Funnels.

MECHANICAL: H. Brine Flotation I. Salt & Hollick Flotation J. Heath & Edwards Flotation K. Aucamp Grease Film.

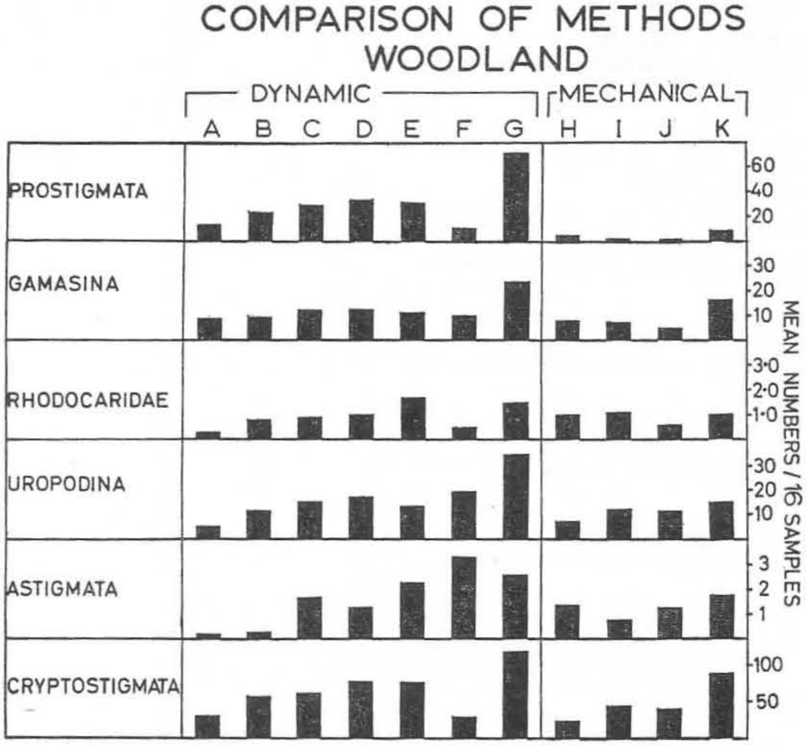

FIG. 7

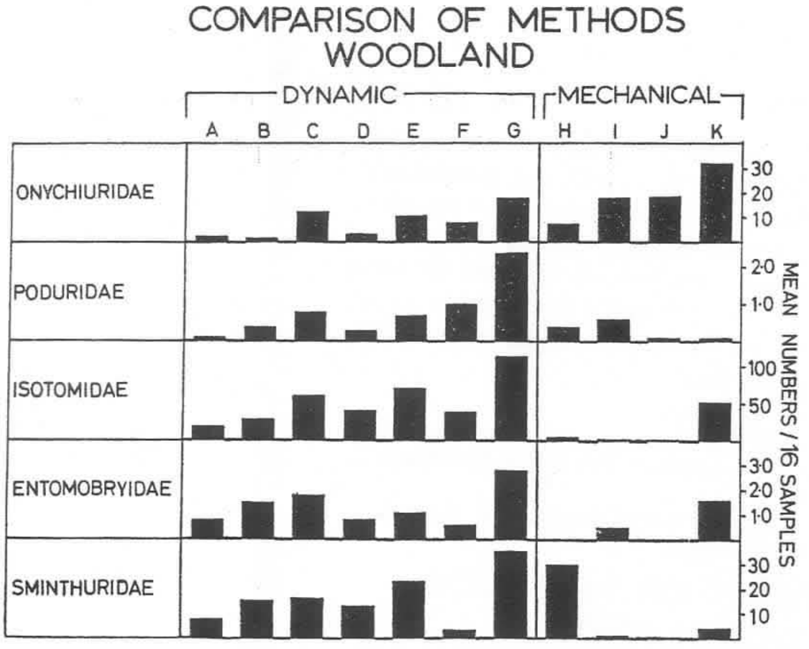
COMPARISON OF METHODS
WOODLAND
DYNAMIC
MECHANICAL
A
B
C
D
E
F
G
H
I
J
K
ONYCHIURIDAE
PODURIDAE
ISOTOMIDAE
ENTOMOBRYIDAE
SMINTHURIDAE
30
20
10
2·0
1·0
100
50
3·0
2·0
1·0
30
20
10
MEAN NUMBERS / 16 SAMPLES

FIG. 8

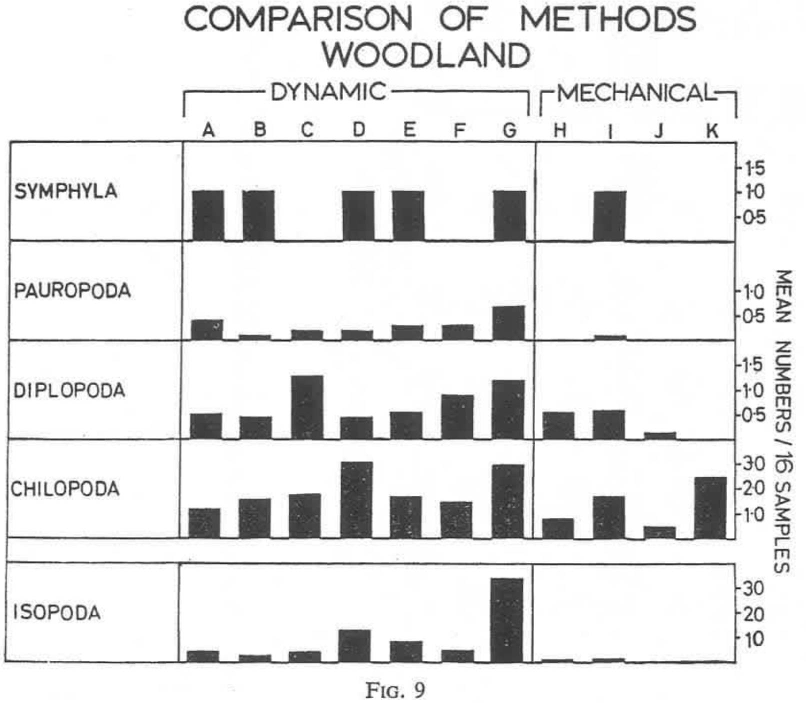
COMPARISON OF METHODS
WOODLAND
DYNAMIC
MECHANICAL
A
B
C
D
E
F
G
H
I
J
K
SYMPHYLA
PAUROPODA
DIPLOPODA
CHILOPODA
ISOPODA
1·5
1·0
0·5
1·0
0·5
1·5
1·0
0·5
3·0
2·0
1·0
30
20
10
MEAN NUMBERS / 16 SAMPLES

FIG. 9

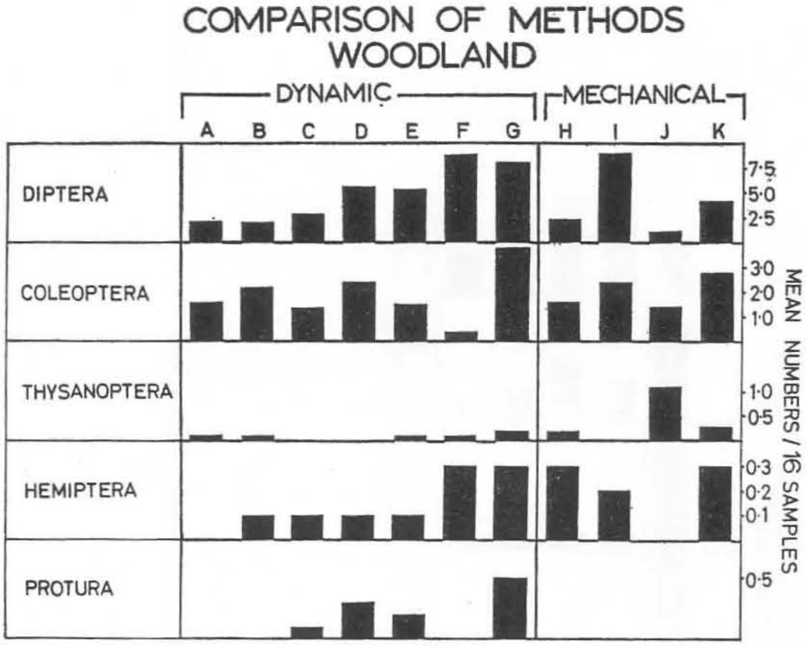
COMPARISON OF METHODS
WOODLAND
DYNAMIC
MECHANICAL
A
B
C
D
E
F
G
H
I
J
K
DIPTERA
COLEOPTERA
THYSANOPTERA
HEMIPTERA
PROTURA
7·5
5·0
2·5
3·0
2·0
1·0
1·0
0·5
0·3
0·2
0·1
0·5
MEAN NUMBERS / 16 SAMPLES

Fig. 10

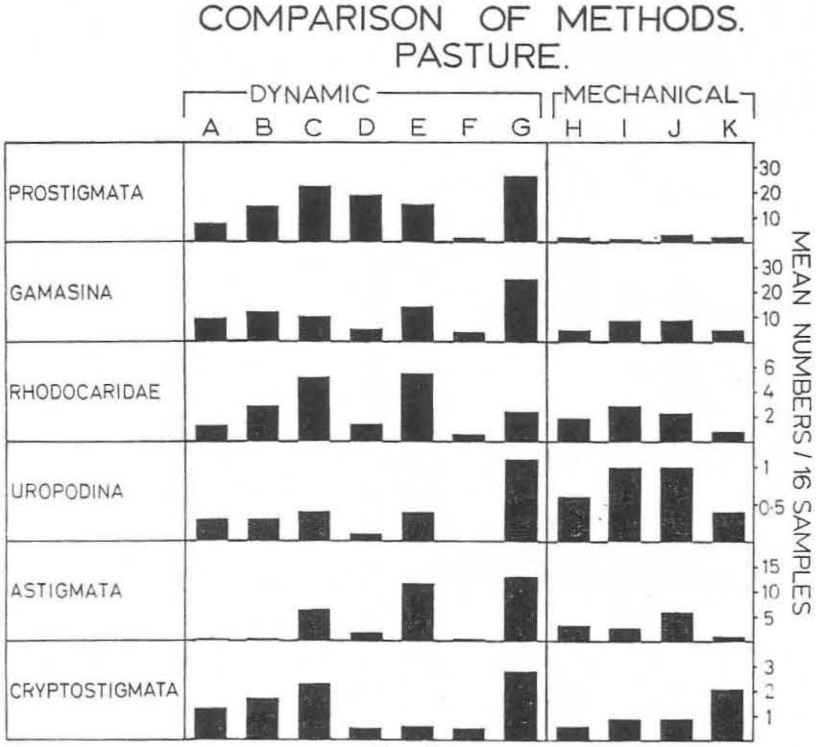
COMPARISON OF METHODS.
PASTURE.
DYNAMIC
MECHANICAL
A
B
C
D
E
F
G
H
I
J
K
PROSTIGMATA
GAMASINA
RHODOCARIDAE
UROPODINA
ASTIGMATA
CRYPTOSTIGMATA
30
20
10
30
20
10
6
4
2
1
0·5
15
10
5
3
2
1
MEAN NUMBERS / 16 SAMPLES

Fig. 11

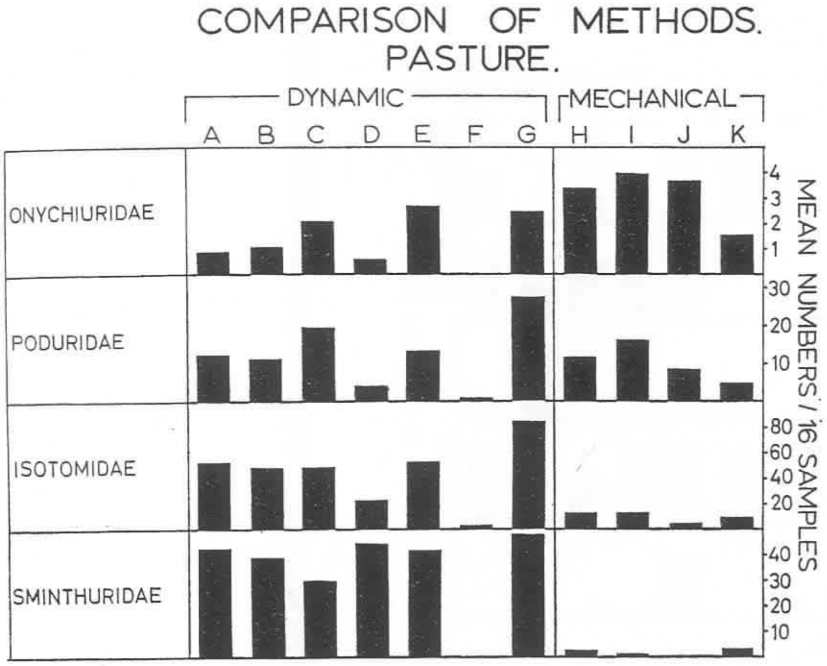
COMPARISON OF METHODS.
PASTURE.
DYNAMIC
MECHANICAL
A B C D E F G H I J K
ONYCHIURIDAE
PODURIDAE
ISOTOMIDAE
SMINTHURIDAE
4 3 2 1
30 20 10
80 60 40 20
40 30 20 10
MEAN NUMBERS / 16 SAMPLES

FIG. 12

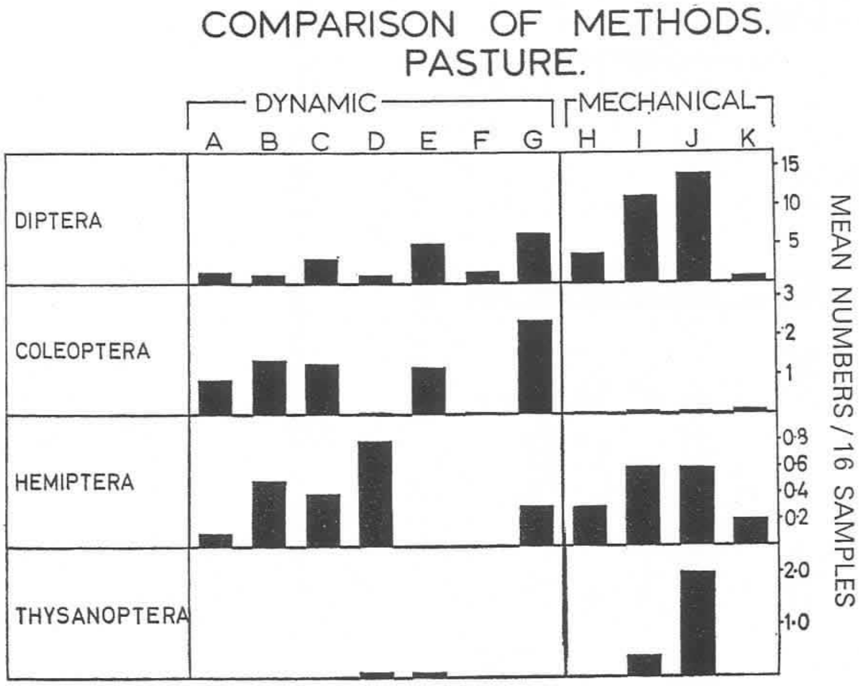
COMPARISON OF METHODS.
PASTURE.
DYNAMIC
MECHANICAL
A B C D E F G H I J K
DIPTERA
COLEOPTERA
HEMIPTERA
THYSANOPTERA
15 10 5
3 2 1
0·8 0·6 0·4 0·2
2·0 1·0
MEAN NUMBERS / 16 SAMPLES

FIG. 13

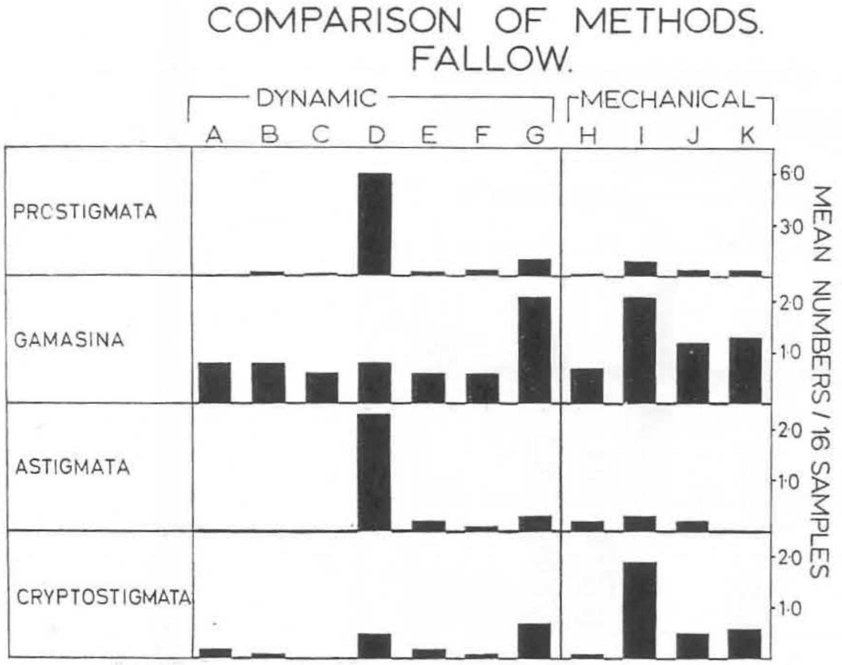
COMPARISON OF METHODS.
FALLOW.
DYNAMIC
MECHANICAL
A
B
C
D
E
F
G
H
I
J
K
PROSTIGMATA
GAMASINA
ASTIGMATA
CRYPTOSTIGMATA
6·0
3·0
2·0
1·0
2·0
1·0
2·0
1·0
MEAN NUMBERS / 16 SAMPLES

FIG. 14

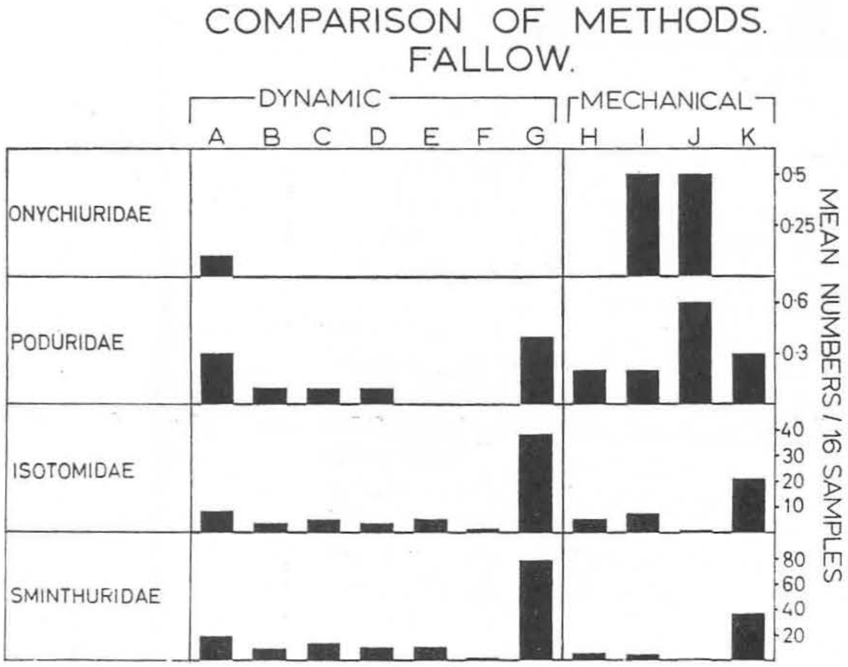
COMPARISON OF METHODS.
FALLOW.
DYNAMIC
MECHANICAL
A
B
C
D
E
F
G
H
I
J
K
ONYCHIURIDAE
PODURIDAE
ISOTOMIDAE
SMINTHURIDAE
0·5
0·25
0·6
0·3
40
30
20
10
80
60
40
20
MEAN NUMBERS / 16 SAMPLES

FIG. 15

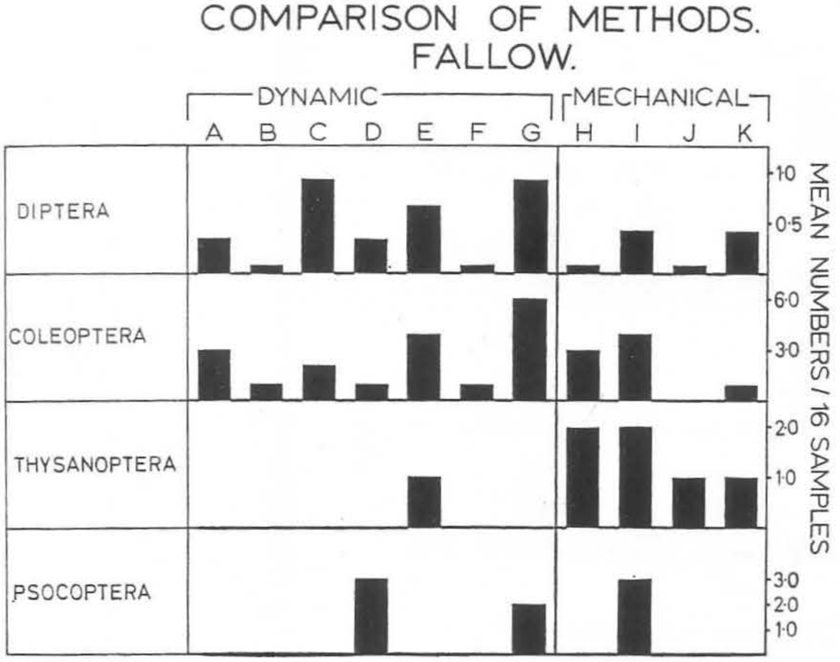

Fig. 16

The animals, extracted from the samples by different methods, were stored in 70% aqueous ethyl alcohol with 5% glycerol, before being sorted into taxonomic classes. In this stage of the investigation 576 samples each containing 50—1000 animals were extracted and the animals identified as shown in Figs. 7–16. The numbers of animals from the small samples were multiplied by volume conversion factors to allow methods to be compared.

Animals are not usually randomly distributed in soil and there were large numbers of some groups in a few samples. All the data were transformed to $\log(n+1)$, and an analysis of variance for the factors, site, method and species revealed highly significant differences between sites, methods and species, and also for the interactions. Woodland soil had most animals, and the arable fewest. No method extracted all animals and absolute efficiency is not known, but relative efficiency for different groups of animals was tested with Tukey's (1953) *w*-procedure, which involves the calculation of an 'honestly significant difference' (hsd) based on the standard error of a treatment mean multiplied by a factor obtained from a table of the 'Upper Percentage Points of the Studentized Range'. The comparisons are summarized for the three habitats in Tables I, II and III.

Mechanical methods, thought to be the more efficient on heavy clay soils, were less efficient for these soils than the best dynamic methods; but they were as efficient as, or more so than, the dynamic methods, only on the fallow site probably because there was less organic matter to interfere with the flotation or to become stuck in the grease-film. The Macfadyen air-conditioned funnels extracted more Collembola and Acarina from both woodland and pasture soils than any of the other methods, except that the onychiurid Collembola were more efficiently extracted by flotation methods.

More myriapods were extracted by funnels than by mechanical methods, except from fallow soil where flotation was better for millipedes and centipedes. Flotation methods were generally better for the larger arthropods, particularly Insecta but both flotation and funnel methods were fairly efficient for these groups. The most efficient method for all groups of animals was the Macfadyen air-conditioned funnel apparatus but the grease-film method was promising for microarthropods although they were not recovered in good condition for taxonomic study and only small soil samples could be used.

V

A comparison of flotation and dynamic methods on different soil types

In Section IV eleven methods were compared with soil from three habitats on a single soil type. The efficiency of a particular method differed with soil, with different amounts of organic matter, and under different types of plant cover. This differing efficiency of extraction with soil of different types was also tested with the Rothamsted pattern Tullgren funnels and Salt and Hollick flotation method, representative of dynamic and mechanical methods. Sixteen pairs of adjacent soil samples 10 cm diam. and 5 cm deep were taken at random from small uniform areas on each of five sites all with different soils, and where possible from more than one habitat on each soil type. One sample from each pair was extracted (intact and inverted) on the Rothamsted pattern funnels with heat applied; the other was frozen at − 10° C and the animals later extracted by the Salt and Hollick technique described previously.

The results, summarized in Table IV, agree closely in many respects with those obtained when eleven methods were compared in Section IV. For all

TABLE II. Comparison of methods—pasture, silt clay loam soil.

Animal Group	Simple Plastic Funnel	Rothamsted Funnels (No Heat)	Rothamsted Funnels (Heat)	Murphy Split Funnels	Kempson Infra-red Extractor	Macfadyen High Gradient Canister	Macfadyen Air Conditioned Funnels	Simple Brine Flotation	Salt and Hollick Flotation	Edwards and Heath Flotation	Grease Film Extractor	Significance of F
Isopoda	—	—	—	—	—	—	H	—	—	—	—	—
Pauropoda	—	H	—	—	—	—	—	—	—	—	—	—
Prostigmata	*	—	—	—	—	*	H	*	*	*	*	0·01
Mesostigmata (Gamasina)	—	—	—	*	—	*	H	*	—	—	*	0·01
Mesostigmata (Uropodina)	—	—	—	—	—	*	H	—	—	—	—	
Astigmata	*	*	—	*	—	*	H	*	*	—	*	0·01
Cryptostigmata	—	—	—	*	—	*	H	—	—	—	—	0·05
Onychiuridae	—	—	—	*	—	*	—	—	H	—	—	0·01
Poduridae	—	—	—	*	—	*	H	—	—	*	*	0·01
Isotomidae	—	—	—	*	—	*	H	*	*	*	*	0·01
Entomobryidae	—	—	—	—	—	—	H	—	H	—	—	—
Sminthuridae	—	—	—	—	—	*	H	*	*	*	*	0·01
Protura	—	—	—	—	—	—	—	—	H	—	—	—
Psocoptera	—	—	—	—	—	—	—	—	—	H	—	—
Hemiptera	—	—	—	H	*	*	—	—	—	—	—	0·05
Coleoptera	*	—	—	*	—	*	—	*	*	*	H	0·01
Diptera	*	*	*	*	—	*	—	*	—	H	*	0·01
Thysanoptera	*	*	*	*	*	*	*	*	—	H	*	0·01

H = Greatest numbers recovered, on average.
— = Not significantly less than H at 5% probability level.
* = Significantly less than H at 5% probability level.

TABLE III. Comparison of methods—fallow, silt clay loam soil

Animal Group	Simple Plastic Funnel	Rothamsted Funnels (No Heat)	Rothamsted Funnels (Heat)	Murphy Split Funnels	Kempson Infra-red Extractor	Macfadyen High Gradient Canister	Macfadyen Air Conditioned Funnels	Simple Brine Flotation	Salt and Hollick Flotation	Edwards and Heath Flotation	Grease Film Extractor	Significance of F
Symphyla	—	—	—	—	H	—	—	—	—	—	—	—
Pauropoda	—	—	H	—	—	—	—	—	—	—	—	—
Diplopoda	—	—	—	—	H	—	—	H	—	—	—	—
Chilopoda	—	—	—	—	—	—	—	—	H	—	—	—
Prostigmata	*	*	*	H	*	*	*	*	*	*	*	0·01
Mesostigmata (Gamasina)	—	—	—	—	—	—	—	—	H	—	—	0·05
Mesostigmata (Uropodina)	—	—	—	—	—	—	—	—	H	—	—	—
Astigmata	*	*	*	H	*	*	*	*	*	*	*	0·01
Cryptostigmata	*	*	*	*	*	*	—	*	H	*	*	0·01
Onychiuridae	*	*	*	*	*	*	*	*	H	H	*	0·01
Poduridae	—	—	—	—	*	*	—	—	—	H	—	0·05
Isotomidae	*	*	*	*	*	*	H	*	*	*	—	0·01
Entomobryidae	—	—	H	—	—	—	—	—	—	—	—	—
Sminthuridae	*	*	*	*	*	*	H	*	*	*	—	0·01
Psocoptera	—	—	—	H	—	—	—	—	—	—	—	—
Hemiptera	—	—	—	—	—	—	—	—	—	—	H	—
Coleoptera	—	—	—	—	—	—	H	—	—	—	—	—
Diptera	—	—	—	—	—	—	H	—	—	—	—	0·01
Thysanoptera	—	—	—	—	—	—	—	—	H	—	—	—

H = Greatest numbers recovered on average.
— = Not significantly less than H at 5% probability level.
* = Significantly less than H at 5% probability level.

Table IV. Comparison of methods on different soil types.

Soil Type:	Peat			Clay Mull Over Limestone	Clay Loam		Silt Clay Loam			Sand Loam		
Habitat:	Woodland	Pasture	Fallow	Woodland	Pasture	Fallow	Woodland	Pasture	Fallow	Woodland	Pasture	Fallow
Animal Group												
Symphyla	0	0	0	—	—	—	0	0	0	0	0	0
Pauropoda	0	0	0	—	—	—	—	0	0	0	—	0
Diplopoda	0	0	0	—	—	—	—	0	0	0	0	0
Chilopoda	$\overset{**}{\text{(F)}}$	0	—	—	—	—	—	0	0	—	0	—
Prostigmata	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{*}{\text{(F)}}$	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$
Mesostigmata (Gamasina)	$\overset{**}{\text{(F)}}$	$\overset{**}{\text{(T)}}$	—	—	—	—	$\overset{**}{\text{(T)}}$	—	—	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$
Mesostigmata (Uropodina)	$\overset{**}{\text{(F)}}$	—	0	—	0	—	—	—	0	—	—	0
Astigmata	$\overset{**}{\text{(F)}}$	$\overset{*}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	—	0	$\overset{**}{\text{(F)}}$	—	—	—	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$
Cryptostigmata	$\overset{*}{\text{(F)}}$	—	$\overset{**}{\text{(T)}}$	—	—	$\overset{*}{\text{(F)}}$	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(F)}}$	$\overset{*}{\text{(T)}}$	$\overset{*}{\text{(T)}}$	—
Onychiuridae	$\overset{**}{\text{(F)}}$	—	$\overset{**}{\text{(F)}}$	—	$\overset{**}{\text{(F)}}$	$\overset{**}{\text{(F)}}$	—	—	—	—	—	$\overset{**}{\text{(T)}}$
Poduridae	$\overset{*}{\text{(F)}}$	$\overset{*}{\text{(F)}}$	0	$\overset{*}{\text{(T)}}$	$\overset{*}{\text{(F)}}$	—	—	—	—	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(T)}}$
Isotomidae	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	—	—	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	—	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$
Entomobryidae	$\overset{*}{\text{(T)}}$	—	0	—	$\overset{*}{\text{(F)}}$	—	0	0	0	—	$\overset{*}{\text{(T)}}$	0
Sminthuridae	0	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	$\overset{*}{\text{(T)}}$	0	$\overset{**}{\text{(T)}}$	—
Hemiptera	—	—	$\overset{**}{\text{(F)}}$	$\overset{*}{\text{(F)}}$	$\overset{**}{\text{(F)}}$	0	0	0	0	—	—	0
Thysanoptera	—	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(F)}}$	—	—	0	0	—	$\overset{*}{\text{(F)}}$	$\overset{**}{\text{(T)}}$	$\overset{*}{\text{(T)}}$
Diptera	$\overset{**}{\text{(T)}}$	—	—	$\overset{**}{\text{(F)}}$	—	—	—	$\overset{**}{\text{(F)}}$	—	$\overset{**}{\text{(F)}}$	$\overset{*}{\text{(F)}}$	—
Coleoptera	—	$\overset{**}{\text{(T)}}$	$\overset{**}{\text{(T)}}$	—	$\overset{**}{\text{(F)}}$	$\overset{*}{\text{(F)}}$	—	—	—	$\overset{*}{\text{(F)}}$	—	—
Protura	0	0	0	—	0	—	0	0	0	—	0	0

— = No significant difference between Means at 5% and 1% levels.
* = Significant difference between Means at 5% level.
** = Significant difference between Means at 1% level.
(T) = Tullgren significantly more efficient.
(F) = Flotation significantly more efficient.
0 = Not known.

the mineral soils the flotation method usually extracted more insects and their larvae but from peat soil, Tullgren methods extracted more. The efficiency of flotation and funnel methods did not differ significantly for many soils and insect groups.

More Collembola and Acarina were extracted by the funnel method from most soils, but with fallow soils the flotation method was more efficient, probably because there is less organic matter in such soil. In contrast to other microarthropods, the less active onychiurid and podurid Collembola tended to be extracted more efficiently by flotation, probably because they are trapped in the soil during funnel extraction. In general, funnel methods are preferable for studying populations of micro-arthropods except with soils containing little organic matter, and flotation methods are preferable for Insecta and larger arthropods, except in soils with much organic matter.

VI

Storage of soil samples

The animals should be extracted from soil soon after the samples are taken but often this may be impossible. The animals can be satisfactorily extracted mechanically even after storage at −10°C for several months. When storing samples for extraction in funnels all the animals must be kept alive, but their numbers change as eggs hatch and individuals die. Ideally all samples should be extracted the day they were taken but if this is impossible changes that occur during storage must be known. Consequently these were assessed.

Many cores, 5 cm diameter, 15 cm deep, were taken very close together from pasture on a clay loam soil. They were mixed randomly and divided into four equal batches and kept at 5, 10, 15 and 20°C. Sixteen samples were also extracted in Rothamsted pattern funnels immediately after they were brought in from the field and a set of sixteen stored samples, from each temperature, extracted in funnels each week thereafter. Results are now complete only for the control samples and those stored at 5° and 20°C for 28 and 56 days; Figs. 17 and 18 summarize the results. After storage for 28 days at 5°C only rhodacarid mites showed a significant ($P = 0{\cdot}05$) decrease in numbers. By 56 days numbers of gamasid mites and Diptera larvae had increased significantly, the larvae presumably from hatched eggs already in the soil. Numbers of Thysanoptera had decreased significantly after 56 days. There were slightly fewer Collembola after storage probably because predatory gamasid mites increased (Edwards, 1967a).

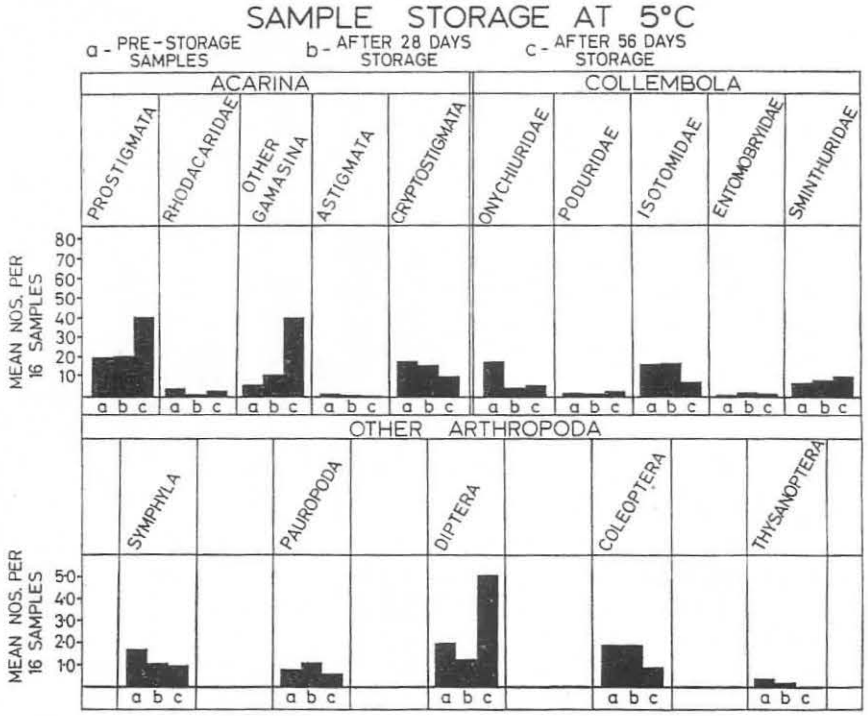

FIG. 17

In samples stored at 20°C, numbers of animals changed much more. Gamasid and rhodacarid mites had increased very rapidly after 28 days and by 56 days there were significantly more astigmatid mites. Numbers of Sminthuridae had lessened significantly by 28 days and those of Symphyla by 56 days. Numbers of prostigmatid mites and enchytraeid worms also increased but not significantly. These results show that storage for up to a week at temperatures of about 5°C should not cause any serious changes in numbers of animals in the samples.

VII

Factors affecting the efficiency of Tullgren funnels

The questionnaires indicated that most soil ecologists use some form of Tullgren funnel and our studies show that the more sophisticated funnel-extraction methods are more efficient than mechanical methods and provide

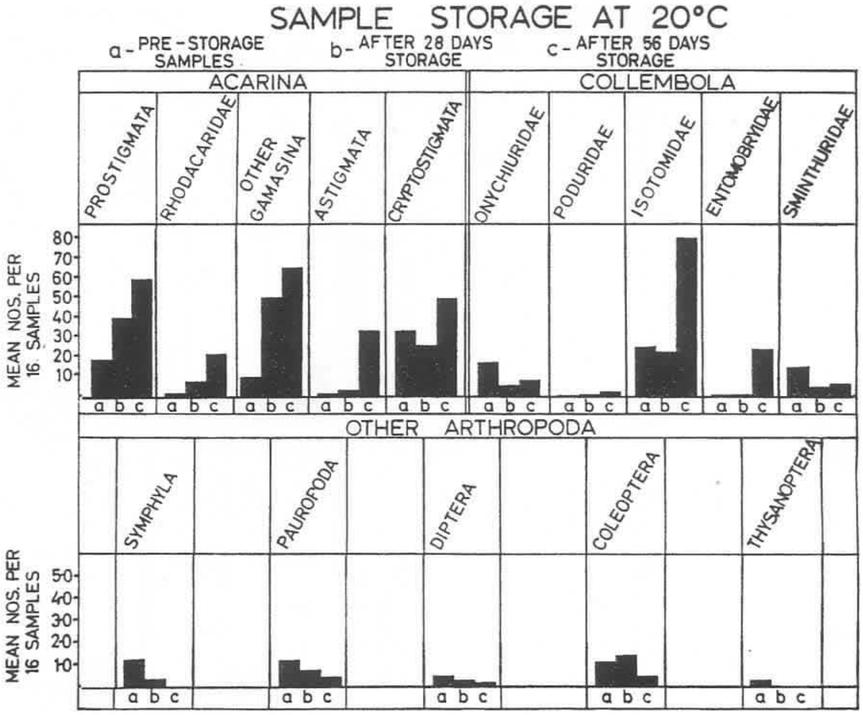

FIG. 18

better material for taxonomic study. It seemed important to study the use, mode of action and construction of funnels in detail, so as to recommend their best use and to suggest improvements in their construction. To do this, factors affecting the efficiency of the Rothamsted-pattern funnels were carefully compared usually with two sets of paired samples, extracting one set in the normal way and varying some factor for the other set. This was augmented by studies of the sequence of extraction of animals in four funnels by means of discs of plastic coated with 'Sticktite', slowly rotating (one revolution in 24 hours) under the outlet from the funnels.

(a) Orientation of the sample on the sieve

Some authors (Murphy, 1962) have suggested that animals can escape better when intact samples are inverted. Many animals live near the surface and these have a shorter distance to travel from an inverted than from an upright

TABLE V. Orientation of samples.

		Intact Inverted V. Crumbled Samples	Intact Inverted V. Intact Upright	Crumbled Samples V. Intact Horizontal	Double Sample V. Single Sample
Prostigmata		**	**	—	—
Mesostigmata	Gamasina	*	**	—	* (D)
	Uropodina	0	—	—	—
Cryptostigmata		—	—	—	—
Onychiuridae		—	—	—	* (S)
Poduridae		—	*	—	* (D)
Isotomidae		—	*	—	** (D)
Entomobryidae		—	—	—	—
Sminthuridae		**	*	—	** (D)
Diptera		**	*	*	** (S)
Coleoptera		**	*	—	—
Thysanoptera		*	**	—	—

** First method significantly better 1% level.
* First method significantly better 5% level.
— No significant difference.
0 No animals.
(S) Single sample better.
(D) Double sample better.

sample. The following tests were therefore made: (i) sets of samples were extracted in upright and inverted positions, (ii) samples were crumbled apart and left intact and inverted during extraction, (iii) cores were extracted horizontally in the sieve and from crumbled samples.

The results are summarized in Table V. More animals were extracted from clay loam when the sample was placed on the sieve intact and inverted, than when crumbled or extracted upright.

(b) Size of sample

The depth of soil in the sieve may affect the efficiency of extraction, for animals may remain trapped in the upper part of the sample. If the sample is too shallow it may dry out too rapidly and animals may die before leaving the soil. A steep gradient of moisture and temperature through the sample seems to increase the efficiency of extraction but it is impossible to maintain a gradient with shallow samples.

(i) *Broken samples*

The effect of the depth of sample on efficiency of extraction was investigated by taking 16 sets of three soil samples 10 cm diam. × 5 cm deep from a small uniform area of pasture on clay loam soil. Two samples from each set of three were broken up and thoroughly mixed together then placed on the sieve of one funnel. The third was broken up and placed on the sieve in another funnel. This was repeated for all samples so that 16 funnels contained single samples and 16 had double samples.

The animals were extracted and sorted into categories then the numbers obtained from the double samples were halved before statistical analysis. Significant differences are summarized in Table V. Gamasid mites, podurid and isotomid Collembola and Psocoptera were extracted most efficiently from the double depth samples, but more onychiurid Collembola and Diptera larvae were recovered from single-depth samples.

Perhaps the more active animals move right through the greater depth of soil and respond better to a temperature and moisture gradient than less active ones, for the more sluggish fly larvae and deep-living Collembola were trapped in the double samples. Sections IV and V also demonstrate that dipterous larvae, and onychiurid Collembola are usually extracted better by flotation than by funnel methods. For most purposes the deeper soil sample appears to be preferable to a shallow one.

(ii) *Intact samples*

To compare the effect of depth of soil on the sieve using intact soil samples, 16 sets of three soil samples 10 cm diam. × 5 cm deep and 16 sets of three 5 cm diam. × 5 cm deep were taken as in the last section. One sample from each set was inverted on a sieve; another was sliced horizontally into two cores 2·5 cm deep and the two half-cores were inverted in two other funnels; the third was sliced horizontally into four sections each 1·25 cm deep and these placed in four more funnels. After the animals from a single original soil sample were extracted, they were bulked together and sorted into taxonomic categories. The sectioned samples tended to yield more Acarina but fewer Collembola than the unsectioned ones, but none of the differences was significant at the 5% probability level. More onychiurid Collembola and dipterous larvae were obtained from the sectioned samples than the unsectioned ones, confirming the results obtained with broken samples.

(c) Collecting fluids

Macfadyen (1962) postulated that the collecting fluid should be odourless, non-repellent and non-volatile and should kill the animals falling into it. The questionnaires indicated that most workers use ethyl alcohol although a few favoured water or other fixatives; picric acid was recommended for use in the Kempson extractor. To test the effect of water, ethyl alcohol and picric acid on the efficiency of extraction, sixteen sets of three 10 cm diam. × 5 cm deep samples were taken from pasture on clay loam soil. Using Rothamsted type funnels one of each set of three was extracted into 70% ethyl alcohol* with 5% glycerol, one into picric acid and the other into distilled water. Table VI shows that many more animals were collected when extracted over picric acid and alcohol than over distilled water, except that dipterous larvae were recovered better over distilled water and picric acid than over ethyl alcohol.

The possibility that animals were escaping from the distilled water because they were not killed was tested by comparing two sets of sixteen samples, one collected into ordinary vials with distilled water and the other into vials with 'Fluon' painted on to the inside rim to prevent arthropods escaping, but there was no significant difference between the two.

It seemed that the alcohol and picric acid might attract animals and hence increase efficiency. This was tested by extracting four sets of sixteen samples, one into vials containing 70% alcohol and 5% glycerol, one into vials with distilled water but with small side-arms containing 70% ethyl alcohol, a third into distilled water and the fourth into distilled water containing a detergent, 'Teepol'. Significantly more Prostigmata, Gamasina, Onychiuridae, Sminthuridae, Thysanoptera and Hemiptera fell into tubes either with alcohol or into water with alcohol in the side arms than into water alone. Although more of other groups were also extracted in the tubes of alcohol, these increases were not significant. The only exception was dipterous larvae, numbers of which were significantly more in samples collected into distilled water. When ethyl alcohol was used as a collecting fluid, fewer animals were extracted with the Macfadyen canister apparatus (Macfadyen, 1962a) than when water was used. This was probably because in this apparatus the collecting fluid is completely enclosed just beneath the sample and the more concentrated vapours affect the efficiency of the extraction. For funnel methods ethyl alcohol or picric acid are probably best.

* Ethyl alcohol=Industrial methylated spirits, containing ⩾ 5% wood naphtha.

TABLE VI.

	70% Alcohol + 5% Glycerol	Picric Acid	Distilled Water
Prostigmata	**	**	—
Gamasina	**	—	—
Cryptostigmata	**	**	—
Onychiuridae	—	**	—
Poduridae	—	—	—
Isotomidae	**	**	—
Entomobryidae	—	—	—
Sminthuridae	**	**	—
Diptera	—	**	**
Coleoptera	—	—	—
Thysanoptera	—	—	—

** = Means significantly greater than —. ($P = 0{\cdot}01$).

VIII

Conclusions and recommendations of the best methods

The results of our experiments enable recommendations to be made about methods for extracting the different groups of arthropods from samples of soil. For productivity studies in the International Biological Programme, arthropod populations in selected sites will need to be accurately assessed and, because the productivity of ecosystems may be compared, methods for assessing populations should be standardized. Facilities, soils, fauna and research workers differ and rigid recommendations are impracticable. But it must be emphasized that before the arthropod populations in any particular site are properly assessed, extensive preliminary comparisons of the effectiveness of different extraction methods for that site, habitat, soil and climate should be made.

Our results indicate broadly the most preferable methods, but do not eliminate the need for tests in particular sites.

No single extraction method or size of sample is best for all groups of animals, and it may be necessary to use several methods and sample sizes. Alternatively where a single method is used for most of the estimations, its efficiency for each group of animals in that particular site should be investigated before it is used extensively. An efficiency correction factor can then be found for modifying estimates obtained with the technique.

TABLE VII. Recommended methods for extracting of soil arthropods

Soil type:	Peat			Clay or Loam			Sand		
Habitat:	Woodland	Pasture	Arable	Woodland	Pasture	Arable	Woodland	Pasture	Arable
Arthropod Group									
Isopoda	AC	ABC	ABC	AC	ABC	ABC	X	X	X
Pauropoda	ABC	ABC	ABC	ABC	ABC	X	X	X	X
Symphyla	ABC	ABC	ABC	X	ABC	X	X	X	X
Diplopoda	ABC	ABC	ABC	ABC	ABC	X	X	X	X
Chilopoda	ABC	ABC	ABC	X	ABC	X	X	X	X
Prostigmata	AC	ABC	AC	AC	ABC	ACD	ABC	ABC	ABC
Gamasina	ABC	ABC	ABC	ABC	ABCDE	X	X	X	X
Uropodina	AC	ABC	ABC	AC	X	X	X	X	X
Astigmata	ABC	ABC	AC	ABC	ABC	AC	ABC	ABC	ABC
Cryptostigmata	ABC	ABC	AC	ABCF	X	ACD	ABCF	X	X
Pseudo-Scorpionida	ABC	ABC	ABC	ABC	ABC	ABC	X	X	X
Aranei	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	X
Onychiuridae	AC	ABC	AC	ACDEF	X	ACDEF	X	X	X
Poduridae	AC	ABC	ABC	AC	ABCD	X	ABCD	ABCD	ABCD
Isotomidae	ABC	ABC	ABC	ABC	ABC	ABCD	ABCD	ABCD	ABCD
Entomobryidae	ABC	ABC	ABC	ABC	ABC	ABCD	ABCD	ABCD	ABCD
Sminthuridae	ABC	ABC	ABC	ABC	ABC	ABCD	ABCD	ABCD	ABCD
Protura	ABC	ABC	ABC	ABC	X	X	X	X	X
Psocoptera	ABC	ABC	ABC	X	X	X	X	X	X
Thysanoptera	ABC	ABC	ABC	DE	DE	X	X	X	X
Hemiptera	ABC	ABC	ABC	X	X	X	X	X	X
Hymenoptera	ABC	ABC	ABC	X	X	X	X	X	X
Coleoptera	ABC	ABC	ABC	X	X	X	X	CDEF	CDEF
Diptera	ABC	ABC	ABC	X	X	X	X	CDEF	CDEF
Eggs and Pupae	DEF	DEF	DEF	DEF	DEF	DEF	DEF	DEF	DEF

A. Simple funnel with heat.
B. Kempson Infra-red extractor.
C. Macfadyen air-conditioned funnel.
D. Salt & Hollick flotation.
E. Edwards & Heath flotation.
F. Grease film extractor.
X. All methods suitable.

Our study indicates that the best general method is a Tullgren funnel of the Macfadyen type, with a steep gradient of temperature and moisture through the sample. A simpler form of funnel of the Rothamsted pattern performed well for most groups of animals. To obtain eggs, pupae or animals in diapause, a mechanical or flotation method is essential. The way the funnels are used is also important. They should be kept in a well-ventilated constant temperature room and thoroughly cleaned between use. For most soils, cores should not be deeper than 5 cm or shallower than 2 cm, and they should be inverted in the sieve containers. The animals are best collected into either picric acid or 70% alcohol with 5% glycerol. Where possible, samples should be placed on the funnels the same day they are collected, but storage at 5°C for up to a week should not greatly affect the results.

Table VII lists the best methods for different groups of soil arthropods. In general, flotation methods are best with sandy soils but of less use in peat or muck soils with much organic matter. For most soils, funnels are most efficient for microarthropods but flotation is often preferable for the larger arthropods. Myriapods are extracted as well by funnels as by flotation from most soils, but their comparatively small numbers mean that large samples (10 cm diameter or greater) are needed. Gamasid and oribatid mites can be extracted efficiently by most methods, although funnels are usually preferable. Prostigmata and sometimes Astigmata are recovered very inefficiently by flotation, and funnels are recommended for them.

Most Collembola are more efficiently extracted by funnels than by flotation, except for the Onychiuridae which are better obtained by flotation. Other Insecta, particularly Diptera and Thysanoptera, are better recovered by flotation methods except from organic soils, but differences between flotation and the better funnel methods are seldom significant.

It must be emphasized that, to assess the numbers of soil arthropods accurately careful preliminary investigation is needed, and that adequate equipment and statistical procedure, very considerable labour and a thorough taxonomic facility are essential.

References

AUCAMP J.L. & RYKE P.A.J. (1964). A preliminary report on a grease film extraction method for soil microarthropods. *Pedobiologia*, **4**, 77–79.

BERLESE A. (1905). Apparecchio per racogliere presto ed in gran numero piccoli artropodi. *Redia*, **2**, 85–89.

Block W. (1966). Some characteristics of the Macfadyen high gradient extractor for soil micro-arthropods. *Oikos*, **17**, 1–9.

Debauche H.R. (1962). The structural analysis of animal communities of the soil. *Progress in Soil Zoology*. Butterworth's, London. 10–25.

Edwards C.A. (1955). Soil sampling for symphylids and a note on populations. *Soil Zoology*. Butterworth's, London. 152–156.

Edwards C.A. (1967a). The effects of gamma irradiation on populations of soil invertebrates. *Proc. Second Nat. Symp. Radioecology, Ann. Arbor, Michigan.* (In press.)

Edwards C.A. (1967b). Relationships between weights, volumes and numbers of soil animals. *Progress in Soil Biology*. Verlag. Friedr. Vieweg & Schn., Braunschweig. 1–10.

Edwards C.A. & Heath G.W. (1962). An improved method for extracting arthropods from soil. *Rep. Rothamsted exp. Stn.* 1962. 159.

Evans G.O. (1951). Investigation on the fauna of forest humus layers. *Report on Forest Research for the year ending March,* 1950. Forestry Commission, London, H.M.S.O. 110–113.

Ford J. (1937). Fluctuations in natural populations of Collembola and Acarina. *J. Anim. Ecol.* **6**, 98–111.

Haarlov N. (1947). A new modification of the Tullgren apparatus. *J. Anim. Ecol.* 115–121.

Hartenstein R. (1961). On the distribution of forest soil microarthropods and their fit to 'contagious' distribution factors. *Ecology*, **42**, 190–194.

Healy M.J.R. (1962). Some basic statistical techniques in soil zoology. *Progress in Soil Zoology*. Butterworth's, London. 3–9.

Hughes R.D. (1962). The study of aggregated populations. *Progress in Soil Zoology.* Butterworth's, London. 51–55.

Kempson D., Lloyd M. & Ghelardi R. (1963). A new extractor for woodland litter. *Pedobiologia*, **3**. 1–21.

Ladell W.R.S. (1936). A new apparatus for separating insects and other arthropods from the soil. *Ann. appl. Biol.* **23**, 862–879.

Macfadyen A. (1953). Notes on methods for the extraction of small soil arthropods. *J. Anim. Ecol.* **22**, 65–77.

Macfadyen A. (1962a). Soil arthropod sampling. *Adv. Ecol. Res.* Academic Press, London and New York. 1–34.

Macfadyen A. (1962b). Control of humidity in three funnel-type extractors for soil arthropods. *Progress in Soil Zoology*. Butterworth's, London. 158–168.

Morris H.M. (1922). On a method of separating insects and other arthropods from soil. *Bull. ent. Res.* **13**, 197–200.

Murphy P.W. (1958). The quantitative study of soil meiofauna. 1. The effect of sample treatment on extraction efficiency with a modified funnel extractor. *Entomologia exp. appl.* **1**, 94–108.

Murphy P.W. (1962). Extraction methods for soil animals. 1. Dynamic methods with particular reference to funnel processes. *Progress in Soil Zoology*. Butterworth's, London. 75–114.

Newell I.M. (1955). An auto-segregator for use in collecting soil-inhabiting arthropods. *Trans. Am. microsc. Soc.* **74**, 389–392.

SALT G. & HOLLICK F.S.J. (1944). Studies of wireworm populations. 1. A census of wireworms in pasture. *Ann. appl. Biol.* **31**, 52–64.

SATCHELL J.E. & NELSON J.M. (1962). A comparison of the Tullgren-funnel and flotation methods of extracting acarina from woodland soil. *Progress in Soil Zoology*. Butterworths, London. 212–216.

SKELLAM J.G. (1962). Estimation of animal populations by extraction processes considered from the mathematical standpoint. *Progress in Soil Zoology*. Butterworth's, London. 26–36.

TUKEY J.W. (1953). *The Problem of Multiple Comparisons*. Princeton University, Princeton, N.J.

TULLGREN A. (1918). Ein sehr einfacher Ausleseapparat fur terricole Tierformen. *Z. angew, Ent.* **4**, 149–150.

ULRICH A.T. (1933). Die Makrofauna der Waldstreu. Quantitativ Untersuchungen in Bestanden mit guter und schlechter Zersetzung des Bestandesabfalles. *Mitt. Forstw. Forstwiss.* **4**, 283–323.

VALLE A. (1951). Su un nuovo sistema di selezione particolarmente adatto per la raccolta in massa dei microarthropodi. *Boll. Zool.* **18**, 299–304.

WOOD T.G. (1965). Comparison of a funnel and a flotation method for extracting Acari and Collembola from moorland soils. *Pedobiologia*, **5**, 131–139.